

AMENDMENTS TO THE SPECIFICATION

Please amend the paragraph beginning at page 1, line 12 as follows:

According to the present invention, the fertilized ovum of an animal can be grown three-dimensionally in a culture system, ~~and thus.~~ As such, the present invention is useful for elucidation of the differences between the three-dimensional growth of the fertilized ovum in an in vitro culture system and the development of the early embryo from the fertilized ovum implanted in vivo[[,]]. In addition, the present invention is useful for the evaluation of teratogenic materials, or grafting of an embryo initially developed from the fertilized ovum, ~~etc.~~

Please amend the paragraph beginning at page 1, line 21 as follows:

Hitherto, ~~there has been established~~ an assisted reproductive technology (ART) has been established, not only in a veterinary field but also in a human sterility treatment, ~~wherein.~~ In this ART a spermatozoon and an ovum are fertilized in vitro in a culture system to prepare a fertilized ovum (a zygote). Then the fertilized ovum can be cultured via cleavage[[,]] (i.e., morula and blastocyst stages to a hatching-blastocyst stage, which is typified by a late blastula stage wherein zona pellucida is denatured and ~~disappeared~~ dissolved)[[,]] and the fertilized ovum at the stages from cleavage to blastula stage [[is]] are transplanted in ~~an a~~ uterus to ~~obtain~~ mature into a baby.

Please amend the paragraph beginning at page 2, line 7 as follows:

~~Also~~ Further, a fertilized ovum (a late blastula) is implanted on an endometrium in vivo and an inner cell mass (an embryoblast) grows to a development stage of early embryo including a gastrula forming process, ~~which.~~ The early embryo then proceeds to grow to a three-layer embryonic disc. However, at present, no reports ~~there are not yet any report~~ of such a growth process in a culture system exists.

Please amend the paragraph beginning at page 2, line 13 as follows:

~~Namely, in~~ In the culture systems hitherto presented, even if a fertilized ovum (a late blastula) is cultured continuously, only monolayer cells are proliferated two-dimensionally. Any three-dimensional architecture having an early embryo-like structure, such that a gastrula or a neurula is produced, has yet not been ~~yet prepared~~ achieved.

Please amend the paragraph beginning at page 2, line 18 as follows:

On one hand, the basic technology of tissue engineering for reconstruction of a tissue from both cultured cells and their scaffold(s) (e.g., a culture carrier(s)) has proceeded eminently for ~~these the~~ the past 10 years centering ~~around~~ in Europe and America. As to organs having relatively simple constructions, ~~the a~~ a basic reconstruction method has been established [Ferber, D., Science 284, 422-425, (1999)].

Please amend the paragraph beginning at page 2, line 24 as follows:

~~Hitherto, in order to construct a tissue by assembling cells and extracellular matrix components three dimensionally, there have been developed carriers having various forms from many materials. Previously, many carriers have been developed having various forms, using many different materials, in order to construct tissues by three-dimensionally assembling cells and extracellular matrix components.~~

Please amend the paragraph beginning at page 3, line 3 as follows:

~~We, the inventors,~~ The present inventors have established the basic technology of tissue engineering ~~with~~ utilizing a mesh network such as cotton gauze as a support (Japanese Patent Application Laid-open No. Hei 7-298876 and Japanese Patent No. 3081130).

Please amend the paragraph beginning at page 3, line 7 as follows:

~~Further~~ In Japanese Patent Application Laid-open No. Hei 11-164684, the inventors ~~have already established~~ describe a novel organ engineering method of reconstructing an organ-like construct (an organoid) by subjecting an organ to continuous three-step perfusion

~~on an organ~~ to remodel the organ into a culture version organoid without separating the majority of constructive cells in the objective organ [(Japanese Patent Application Laid-open No. Hei 11-164684)].

Please amend the paragraph beginning at page 3, line 13 as follows:

~~Further, as to a reconstructing technology of an endometrium, it~~ It has been reported that the endometrial epithelial cells reconstructs a uterine gland-like structure by co-culturing human endometrial epithelial cells and stromal cells in a collagen gel [Akoum, A., et al., J. Reprod. Med., 41, 555-561, (1996)].

Please amend the paragraph beginning at page 3, line 18 as follows:

~~Further, for rabbit, there is a report to culture~~ In rabbits, it has been reported that endometrial epithelial cells can be cultured on a matrigel[[,]] (a reconstituted basement membrane)[[,]] and ~~thereafter to place a blastocyst~~ just before implantation a blastocyst is placed on the matrigel thereon for co-culturing. Although ~~there is described~~ it is disclosed that cell fusion of a trophoblast (cytotrophoblast) with the endometrial epithelial cells occurred at 48 hours after co-culturing [Tominaga Tosirou, Nihon Sanfujinka Gakkai Zasshi, 48, 591-603, (1996)], there is no description that the cells derived from the blastocyst grow to form a three-dimensional architecture having an early embryo-like structure such that a gastrula or that a neurula is formed.

Please amend the paragraph beginning at page 4, line 4 as follows:

~~Namely, there is not yet any report~~ Therefore, at present, no reports exist of a culture carrier or a co-culturing carrier on which a fertilized ovum of an animal is cultured to induce three-dimensional growth.

Please amend the paragraph beginning at page 4, line 8 as follows:

An object of the invention is to provide a carrier for co-culturing a fertilized ovum of an

animal in which behavior of the fertilized ovum of an animal can be easily observed in a culture system and by which adhesion and three-dimensional growth of the fertilized ovum ~~become~~ are possible ~~at first~~.

Please amend the paragraph beginning at page 4, line 13 as follows:

~~Further, another~~ Another object of the invention is to provide a method of culturing the fertilized ovum of an animal, in which the fertilized ovum of an animal can be grown three-dimensionally by culturing the fertilized ovum of an animal using the co-culturing carrier. This method also permits ~~and in which~~ elucidation of the differences between the three-dimensional growth of the fertilized ovum in an in vitro culture system and the development of the early embryo from the fertilized ovum implanted in vivo, evaluation of teratogenic materials, or grafting of an early embryo developed from the fertilized ovum, et cetera ~~etc. become possible~~.

Please amend the paragraph beginning at page 4, line 23 as follows:

In order to develop a carrier for co-culturing a fertilized ovum of an animal and a culturing method of a fertilized ovum of an animal in which: a) behavior of the fertilized ovum of an animal can be easily observed in a culture system; and b) ~~in which~~ adhesion and three-dimensional growth of the fertilized ovum become possible, the inventors have studied ~~eagerly diligently, and found~~ As a result of this study, the inventors have determined that a carrier for co-culturing a fertilized ovum of an animal composed of a cell incorporated type three-dimensionally reconstructed tissue in which cells are beforehand incorporated in a culture carrier makes induction of adhesion and three-dimensional growth of the fertilized ovum possible ~~to complete the present invention based on this founding~~.

Please amend the paragraph beginning at page 5, line 11 as follows:

~~Namely, according~~ According to the first aspect of the invention, ~~there is provided~~ is a carrier for co-culturing a fertilized ovum of an animal comprising a cell incorporated type three-

dimensionally reconstructed tissue for co-culturing the fertilized ovum of an animal to induce adhesion and three-dimensional growth of the fertilized ovum.

Please amend the paragraph beginning at page 5, line 17 as follows:

~~Further, according~~ According to the tenth another aspect of the invention, ~~there is provided~~ is a method of culturing a fertilized ovum of an animal, ~~characterized in that wherein~~ any co-culturing carrier as described in the ~~first to ninth other~~ aspects of the invention is introduced into a culture vessel to culture the fertilized ovum of an animal.

Please amend the paragraph beginning at page 8, line 4 as follows:

~~At first,~~ In the first embodiment of the present invention is a carrier for co-culturing a fertilized ovum of an animal ~~according to the first aspect of the invention is illustrated.~~

Please amend the paragraph beginning at page 8, line 16 as follows:

As a mammal, there may be mentioned human beings, monkey, bovine, sheep, goat, baboon, pig, dog, guinea pig, rat and mouse etc.

Please amend the paragraph beginning at page 8, line 18 as follows:

~~Further, the~~ The fertilized ovum used in culturing may be any stage of a zygote, cleavage, morula or blastocyst stage, but one grown to a blastocyst stage is preferable as an implantation model.

Please amend the paragraph beginning at page 8, line 21 as follows:

~~Further, in~~ In the culture system according to the first aspect of the invention, any ovum in a life cycle other than a fertilized ovum, namely an ovum cell before fertilization such as an ovum in follicle and an ovulated ovum or a fertilizing ovum, may be used.

Please amend the paragraph beginning at page 9, line 6 as follows:

~~Namely, in~~ In addition to the ability that the carrier for co-culturing the fertilized ovum of an animal according to the first aspect of the invention is suitable for adhesion of the

fertilized ovum, ~~not only it cultures~~ as well as to support culturing of the fertilized ovum (blastocyte) as hitherto to proliferate monolayer cells two-dimensionally, but also it can prepare a three-dimensional architecture derived from the fertilized ovum.

Please amend the paragraph beginning at page 10, line 12 as follows:

Similarly, as the cells to be incorporated in the cell incorporated type three-dimensionally reconstructed tissue, cells derived from an ovary or cells derived from ~~an~~ a uterine tube may be used ~~for reflecting~~ to mimic an in vivo environment of about a life cycle of the ovum to be cultured.

Please amend the paragraph beginning at page 10, line 22 as follows:

Such a cell incorporated type three-dimensionally reconstructed tissue is reconstructed from any of cells, tissues or organs derived from animal, and it contains at least one kind of cells as is described in the second aspect of the invention. ~~That is, for~~ For example, ~~[[it]]~~ the cell incorporated type three-dimensionally reconstructed tissue can be obtained by culturing the above-mentioned cells with a culture medium.

Please amend the paragraph beginning at page 11, line 9 as follows:

The cell incorporated type three-dimensionally reconstructed tissue preferably contains an extracellular matrix component and/or a mesh network as is described in the third aspect of the invention. By containing ~~them~~ these components, liquid permeability of the culture medium is improved to culture the incorporated cells effectively and to provide tension on the incorporated cells, whereby three-dimensional growth of the fertilized ovum can be ~~proceeded~~ in a condition more close to a conducted in an environment that mimics the living body.

Please amend the paragraph beginning at page 12, line 6 as follows:

~~Then,~~ Within the context of the present invention, the "mesh network" ~~is referred~~ refers to a fibrous mass having such an opening to form a spatial shape for three-dimensional

culturing, and as is described in the eighth aspect of the invention, there may be mentioned natural or synthetic threads and/or woven masses thereof.

Please amend the paragraph beginning at page 13, line 11 as follows:

~~Further, the~~ The mesh network is preferably bioabsorptive according to the ninth aspect of the invention. The term bioabsorption is ~~referred~~ refers to a property to be absorbed and degraded in a living body. Since it can absorb the culture carrier in a living body, it is quite useful for transplantation, etc.

Please amend the paragraph beginning at page 14, line 2 as follows:

In contrast, by containing the mesh network, contraction of the cell incorporated type three-dimensionally reconstructed tissue is inhibited, so that behavior of the fertilized ovum can be preferably observed by means of a phase-contrast microscope, ~~thus it is preferable.~~

Please amend the paragraph beginning at page 16, line 6 as follows:

The co-culturing carrier according to the first aspect of the invention, which is composed of the above-mentioned cell incorporated type three-dimensionally reconstructed tissue, can be used for culturing of a fertilized ovum.

Please amend the paragraph beginning at page 17, line 2 as follows:

As the culture medium, ~~there may be used those used~~ any medium suitable for preparing a cell incorporated type three-dimensionally reconstructed tissue may be employed. Such culture medium is changed every one to three day (s). The culturing temperature is 37.0-39.0°C and the culturing period is about 1-60 day (s).

Please amend the paragraph beginning at page 17, line 7 as follows:

When the fertilized ovum is cultured by a culturing method according to the tenth aspect of the invention, the behavior of the fertilized ovum during culturing can be observed by means of a phase-contrast microscope, etc. (see Fig. 6-10). In addition, ~~and also~~ the cells derived from

the fertilized ovum are moved around the fertilized ovum to make three-dimensional growth of the fertilized ovum finally possible ~~finally~~ (see Fig. 11-14).

Please amend the paragraph beginning at page 17, line 14 as follows:

~~According to the~~ The first aspect of the invention, ~~there is provided~~ provides a carrier for co-culturing a fertilized ovum composed of a cell incorporated type three-dimensionally reconstructed tissue, and thus, behavior of the fertilized ovum of an animal in a culture system can be observed easily and adhesion and three-dimensional growth of the fertilized ovum can first become possible ~~at first~~.